Factors Affecting the Occurrence of Second Copulation by Mediterranean Fruit Fly Females (Diptera: Tephritidae)

Stephan G. Lee, Susan D. McCombs, and **Stephen H. Saul**
Department of Entomology, University of Hawaii, Honolulu, Hawaii 96822

Abstract. Logistic regression was used to construct two models to predict the occurrence of second copulations by Mediterranean fruit fly females in sequential copulations with fertile males or with irradiated and non-irradiated males. Male genotype and duration of the first copulation were significant variables in determining the occurrence of a second copulation by individual females. Male age and irradiated male mating order were additional significant variables in sequential copulations with irradiated and non-irradiated males.

Two mating strategies of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), have been observed in the field (Prokopy and Hendrichs 1979, Hendrichs et al. 1991). The primary strategy involves organized aggregations of both sexes with interactions between the males for mating arenas and complex male courtship behavior. The other strategy, mating without courtship behavior, typically occurs on host fruits as previously mated females attempt to oviposit (Prokopy and Hendrichs 1979, Burk and Calkins 1983, Hendrichs and Hendrichs 1990). Forced matings are rare because the female is usually successful in spurning the male.

McInnis (1993) found field-collected females which had mated with both sterile and fertile males. The potential impact of these rare multiple copulations on the sterile insect release technique in which several million sterile flies are released is unknown, although limits on the effects can be estimated by simulation or mathematical modeling. We do know that sperm of the second mate has precedence over that of the first for both fertile (Saul et al. 1988, Saul and McCombs 1993a, Lee 1994) and sterile males (Katiyar and Ramirez 1970). This means that a female who mates with a sterile male can regain fertility through acceptance of a second fertile mate and, conversely, mating with a sterile male second can reduce the female's fertility.

Quantification and manipulation of variables to increase the incidence of second copulations for sterile males could lead to increased effectiveness of sterile insect release programs, i.e., by development of a strain whose males have a high success rate of forced matings (Saul and McCombs 1993b).

Materials and Methods

Rearing. Mating trials and rearing (Saul 1982) were conducted in the Genetic Stock and Clone Center for Tephritid Fruit Flies located in a quarantine facility at the Department of Entomology, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu. Flies used were from laboratory colonies of 5-10 generation from wild flies. Egg-to-egg generation time under normal laboratory conditions (23-25∞C, 65-75% RH) was approximately 30 d. Rearing of males for mating trials was as given by Lee (1994).

Remating protocols. Two pupal color traits were used as genetic markers to determine the paternity of progeny. Test females were homozygous for two non-linked recessive genes,

white pupae (Rössler 1979) and dark pupae (Rössler and Koltin 1976) (*w/w; dp/dp*). Males of two genotypes, wild-type (+/+; +/+) and dark pupae (+/+; *dp/dp*), were used. Females mating with wild-type males produced progeny with brown puparia while those progeny from matings with dark pupae males had black puparia.

Females were aspirated singly into cages 24 h before the mating trial. Single males were aspirated into the cages between 0800-0930 to initiate the trial. Three-hundred replicates were used for the mating sequence wild-type first and dark pupae male second and 480 matings pairs for the mating sequence dark pupae first and wild-type second. Copulation was defined as genital contact for at least 15 minutes. Copulating flies were allowed to separate naturally, and then the males were removed. The remating trial was run 24 h after the initial mating trial. The procedure for the remating trial was the same as the initial mating trial, introducing a male of the alternate genotype; i.e., if the first copulation was with a wild-type male, then the dark pupae male was introduced into the cage for the remating trial.

The procedure for sequential matings with irradiated and non-irradiated males differed in that the male and female ages were 4-12 d and one of the two males was irradiated. The copulation rate for irradiated males was lower than that of the fertile males (Lee 1994) requiring more replicates to obtain the minimum of 15 copulations required for the analysis. Two-hundred and forty mating pairs were used for the mating sequence irradiated wild-type male first and dark pupae male second and 200 pairs were used for the reciprocal sequence. Approximately 699 matings were used for the mating sequence wild-type male first and irradiated dark pupae second and 714 pairs for the reciprocal mating sequence.

Statistical techniques. Variables from double matings (Model I) and sequential matings with irradiated and non-irradiated males (Model II) were evaluated with logistic regression (SPSS 1993) to determine the significance of each variable in prediction of a second copulation. The independent variables for both models were: duration of the first copulation; first male genotype; second male genotype; first male age; second male age; female age at first copulation; and female age at second copulation. In the sequential mating with irradiated and non-irradiated males, irradiation status of the first and second male was an additional variable. The dependent variable for both models was the occurrence of the second copulation. The predicted probability values were calculated from the equation

$$\text{Probability (second copulation)} = 1 / (1 + e^{-Z}) \quad (1)$$

$$\text{where } Z = B_0 + B_1X_1 + B_2X_2 + ... + B_pX_p \quad (2)$$

The values for B_p were logistic regression coefficients of the variables used in the model. The values for X_p were the variable values used in the model.

Non-continuous variables having more than two categories, i.e., male age and female age variables, were coded for consideration as separate categories. Copulation duration was considered a continuous variable and the other variables had only two categories, i.e., genotype: 0 = wild-type and 1 = dark pupae.

Predictor variables were selected by a backward stepwise selection process, where all variables were placed in the model and evaluated by the likelihood-ratio test (Noru_is 1993) for inclusion in the model. The significance of each selected variable on the occurrence of a second copulation was tested by the model and an *R* statistic was calculated. The *R* statistic represents the partial correlation between the dependent variable and each of the independent variables and ranges from 1 to -1. A positive *R* indicated that the likelihood of an event occurring increased with an increase of the variable value.

We sought to maximize the number of correctly predicted second copulations and, equally importantly minimize the number of wrongly predicted non-second copulations, which would

tend to invalidate the predictive model. An increase in the number of wrongly predicted copulations was considered more acceptable because variables not considered in the model could negatively affect the occurrence of a second copulation, i.e., environmental disturbances, noise, nutrition, temperature, and humidity. Models assigned a predicted probability to each case with values 0 through 1. Initially, those cases with predicted probabilities above 0.5 were classified as predicted second copulation those 0.5 or below 0.5 were classified as no second copulation. Later the classification rule was lowered to optimize the model, so as to increase the number of correctly predicted events, a valid procedure in logistic regression.

Results and Discussion

Mating experiments. ***Double matings.*** Approximately 300 mating pairs were used for the mating sequence wild-type male first and dark pupae male second. One-hundred and six females copulated with a wild-type male for the first copulation, and 44 (or 42%) of these copulated again after 24 h with a dark pupae male. Approximately 480 mating pairs were used for the mating sequence dark pupae male first and wild-type male second. Three-hundred and twenty-five females copulated with a dark pupae male for the first copulation. A total of 42 females (or 13%) copulated again with a wild type male after a 24 h respite.

There was a significant difference between wild-type males and dark pupae males for duration of the first and second copulations (Table 1). The wild-type male copulated longer in both mating sequences (Table 1). When dark pupae males were the first maters, the duration of the second copulation was significantly longer than the duration of the first copulation. There was no significant difference in copulation duration for the reciprocal cross. Copulation times for mating sequences A and B are close to, or greater than, 2 h, thus 80-99% of the sperm should have been transferred (Seo et al. 1990).

Sequential matings with irradiated and non-irradiated males. Approximately 240 mating pairs were used for the mating sequence irradiated wild-type male first and non-irradiated dark pupae male second. Seventy-six females copulated with an irradiated wild-type male for the first copulation, and 30 (or 39%) of these copulated again after 24 h with a dark pupae male. Approximately 200 mating pairs were used for the mating sequence irradiated dark pupae male first and non-irradiated wild-type male second. Seventy-four females copulated with an irradiated dark pupae male for the first copulation. Twenty of these (or 27%) copulated again with a wild type male after 24 h.

Approximately 699 mating pairs were used for the mating sequence non-irradiated wild-type male first and irradiated dark pupae male second. Three-hundred and fourteen females copulated with a wild-type male for the first copulation. Of these 314 females, 19 (or 6%) copulated with an irradiated dark pupae male after 24 h. Approximately 714 mating pairs were used for the mating sequence non-irradiated wild-type male first and irradiated dark pupae male second. Three-hundred and fifteen females copulated with a dark pupae male for the first copulation. Of these 315 females, 20 (or 6%) subsequently copulated with an irradiated wild-type male after 24 h.

Irradiated wild-type males had significantly longer first copulations than the other mating sequences (Table 1). For mating sequences B, D, and F, all of which have the wild-type male as the second mater, there was a significant difference between duration of first and second copulation, with the second copulation significantly longer. Copulation duration varied greatly between multiple mating sequences (Table 1).

Factors that affect second copulation. ***Double matings.*** Model I predicted the occurrence of a second copulation 24 h after the first copulation in double copulations with fertile males. Significant variables for predicting second copulations were duration of the first

Table 1. Duration of first and second copulations for sequential copulations using irradiated and non-irradiated, wild type and mutant dark pupae males.

Mating sequence	Copulation duration (min)(n)	
	First	Second
A. Wild-type ⇒ dark pupae	153.81a (12.95,42)	150.45a (8.81,42)
B. Dark pupae ⇒ wild-type	117.00b (76.66,39)	189.21a (69.79,39)
C. I Wild-type ⇒ dark pupae	167.13a (13.27,30)	164.47a (11.15,30)
D. I Dark pupae ⇒ wild-type	75.94d (12.85,18)	140.50e (15.81,18)
E. Wild-type ⇒ I dark pupae	105.67e (19.26,18)	139.72f (16.03,18)
F. Dark pupae ⇒ I wild-type	79.68d (11.25,19)	174.00a (14.45,19)

I indicates irradiated male. Mean duration of copulations with the same letter are not significantly different at the P = 0.05 level. Duration of first copulation is significantly different from duration of second copulation for copulation sequences B ($P = 0.000$), D ($P = 0.003$), and F ($P = 0.000$). Difference between mean durations of the first copulation are significantly different for sequences A and B ($P = 0.043$), A and D ($P = 0.001$), A and E ($P = 0.045$), A and F ($P = 0.001$), B and C ($P = 0.008$), B and D ($P = 0.046$), B and F ($P = 0.029$), C and D ($P = 0.000$), C and E ($P = 0.009$), and C and F ($P = 0.000$). Differences between mean durations of second copulations are significantly different for B and D ($P = 0.016$) and B and E ($P = 0.015$).

copulation ($P < 0.0001$, $R = -0.2761$) and first male genotype ($P = 0.0001$, $R = -0.1806$). The predicted probability values (Z) were calculated from equation 1, where

$$Z = -3.6757 - 0.0002(X_1) - 2.8964(X_2) + 6.8586(X_3) - 1.3989(X_4) + 7.0019(X_5) - 1.6923(X_6) + 6.9490(X_7) + 6.9966(X_8) \quad (3)$$

and X_1 = duration of first copulation (duration in seconds), X_2 = first male genotype (0 = wild-type, 1 = dark pupae), X_3 = females 9-10 d of age for first copulation (0 = no, 1 = yes), X_4 = females 10 d of age for first copulation (0 = no, 1 = yes), X_5 = females 10-11 d of age for first copulation (0 = no, 1 = yes), and X_6 = females 11 d of age for first copulation (0 = no, 1 = yes), X_7 = second mater males 12 d of age (0 = no, 1 = yes), and X_8 = second mater males 13 d of age (0 = no, 1 = yes).

The negative R value for duration of the first copulation indicated that as the duration of the first copulation increased, the probability of a second copulation decreased. This was consistent with observations of Saul et al. (1988) who found that longer first mating was negatively correlated with the proportion of females copulating a second time. Females copulated a second time more readily with dark pupae males than with wild-type males as indicated by the negative R value for genotype.

There were a total of 356 first copulations. Of these, 271 females did not copulate a second time and 85 of the females did copulate a second time. When we test each mating individually, Model I predicted 318 of the first copulations would not lead to second copulations and 257 or 80.8% did not lead to second copulations, i.e., were correct predictions. The model predicted 38 second copulations and 24 or 63.2% did lead to second copulations, i.e., were correct . The overall successful prediction rate for Model I is therefore 281 (257 + 24) correct and 75 incorrect. The overall successful prediction rate is therefore 78.9% (Table 2).

Sequential matings with irradiated and non-irradiated males. Model II predicted the occurrence of second copulations for sequential copulations with irradiated and non-irradiated males. Significant variables were the duration of the first copulation ($P < 0.0001$, $R = -0.3294$), first male genotype ($P = 0.0003$, $R = -0.1412$), second male age ($P = 0.0299$, $R = 0.0741$), whether the irradiated male was the first mater or second mater ($P < 0.0001$, $R = -0.3283$), and the constant term ($P < 0.0001$, No R statistic for constant term). The predicted probability values were calculated from equation 1, where

$$Z = 4.1875 - 0.0003(X_1) - 2.1720(X_2) - 1.0150(X_3) + 0.6851(X_4) - 0.3159(X_5) \quad (4)$$

and X_1 = duration of first copulation (duration in seconds), X_2 = irradiated male mating order (1 = first mater, 2 = second mater), X_3 = first male genotype (0 = wild-type, 1 = dark pupae), X_4 = second male maters 6 d of age, (0 = no, 1 = yes), and X_5 = second male maters 9-10 d of age (0 = no, 1 = yes).

R values indicated that longer first copulations discouraged females from accepting a second copulation. The mating sequence wild-type first and dark pupae second was more likely to result in a second copulation than the reciprocal cross. Mating sequences with irradiated males as second maters were less likely to have a second copulation than compared to mating sequences with irradiated males as first maters.

Second male age was significant but the small R value indicated small partial contribution to the model. The second male age had two categories in the model: second male maters 6 d of age ($P = 0.0150$, $R = 0.0845$) and second male maters 9-10 d of age ($P = 0.6323$, $R = 0.0000$). The category second male maters 6 d old was significant, indicating that younger irradiated males were more successful at second copulations than older irradiated males. The constant was significant ($P = 0.0000$) which indicates other factors may significantly affect second copulations.

There were a total of 761 first copulations. Of these, 672 females did not copulate a second time and 89 of the females did copulate a second time. When we test each mating individually, Model II predicted 721 of the first copulations would not lead to second copulations and 654 or 91.1% did not lead to second copulations, i.e., were correct predictions. The model predicted 40 second copulations and 22 or 55.0% did lead to second copulations, i.e., were correct . The overall successful prediction rate for Model II is therefore 676 (654 + 22) correct and 85 incorrect. The overall successful prediction rate is therefore 88.8%. (Table 2)

Predictive models constructed with logistic regression provide a quantitative way to determine the effect of many variables on the occurrence of second copulation. Manipulation of variables to increase the incidence of second copulations for sterile males could lead to increased effectiveness of sterile insect release programs by development of strains in which males have a high success rate in second matings (Saul and McCombs 1993b). This study is preliminary in nature because only a few variables have been considered. The models developed herein can be used as a basis for investigating the effects of other variables, i.e., time of remating, male and female age of wild flies instead of wild type laboratory raised

Table 2. Classification tables for model I and model II showing the correct and incorrect predictions for individual copulations.

Classification Table for Model I

		Predicted	
		No	Yes
Observed		N	Y
No	N	257	14
Yes	Y	61	24

Overall correct prediction rate 78.93%

Classification Table for Model II

		Predicted	
		No	Yes
Observed		N	Y
No	N	654	18
Yes	Y	67	22

Overall correct prediction rate 88.83%

flies. Field cage observations of sterile males could be used to construct predictive models to estimate the effectiveness of sterile insect release programs.

Acknowledgment

This manuscript contains data submitted by S. G. Lee in partial fulfillment of the requirements for the degree of Masters of Science in Entomology at the University of Hawaii at Manoa. Journal Series No. 0000 of the Hawaii Institute of Tropical Agriculture and Human Resources. This research was supported by USDA under CSRS special grant HAW-00763-G.

Literature Cited

Burk, T. and **C. C. Calkins.** 1983. Medfly mating behavior and control strategies. Fla. Entomol. 66: 3-18.

Hendrichs, J., and **M. A. Hendrichs.**. 1990. Mediterranean fruit fly (Diptera: Tephritidae) in nature: location and diel pattern of feeding and other activities on fruiting and nonfruiting hosts and nonhosts. Ann. Entomol. Soc. Am. 83: 632-641.

Hendrichs, J. B., I. Katsoyannos, D. R. Papaj and **R. J. Prokopy,** 1991. Sex differences in movement between natural feeding and mating sites and trade-offs between food consumption, mating success and predator evasion in Mediterranean fruit fly (Diptera: Tephritidae). Oecologia 86: 223-231.

Katiyar, K. P. and **E. Ramirez.** 1970. Mating frequency and fertility of Mediterranean fruit fly females alternately mated with normal and irradiated males. J. Econ. Entomol. 63: 1247-1250.

Lee, S. G. 1994. Sperm precedence and second copulation prediction for the Mediterranean fruit fly (Diptera: Tephritidae): Influence of male irradiation and genotype. M. S. Thesis, University of Hawaii, Honolulu.

McInnis, D. O. 1993. Size differences between normal and irradiated sperm heads in mated female Mediterranean fruit flies (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 86: 305-308.

Noru_is, M. J. 1993. SPSS for Windows advanced statistics release 6.0. SPSS Inc. Chicago IL.

Prokopy, R. J. and **J. Hendrichs.** 1979. Mating behavior of *Ceratitis capitata* on a field-caged host tree. Ann. Entomol. Soc. Am. 72: 642-648.

Rössler, Y. 1979. The genetics of the Mediterranean fruit fly: a 'white pupae' mutant. Ann. Entomol. Soc. Am. 72:583-585.

Rössler, Y. and **Y. Koltin.** 1976. The genetics of the Mediterranean fruit fly, *Ceratitis capitata*: three morphological mutations. Ann. Entomol. Soc. Am. 69: 604-608.

Saul, S. H. 1982. Rosy-like mutant of the Mediterranean fruit fly (Diptera: Tephritidae), and its possible use in a genetic sexing program. Ann. Entomol. Soc. Am. 75: 480-483.

Saul, S. H. and **S. D. McCombs.** 1993a. Dynamics of sperm use in the Mediterranean fruit fly (Diptera: Tephritidae): reproductive fitness of multiple-mated females and sequentially mated males. Ann. Entomol. Soc. Am. 86: 198-202.1993b. Increased remating frequency in sex ratio distorted lines of the Mediterranean fruit fly (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 86: 631-637.

Saul, S. H., S. Y. T. Tam and **D. O. McInnis.** 1988. Relationship between sperm competition and copulation duration in the Mediterranean fruit fly (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 81: 498-502.

Seo, S. T., R. I. Vargas, J. E. Gilmore, R. S. Kurashima and **M. S. Fujimoto.** 1990. Sperm transfer in normal and gamma-irradiated, laboratory-reared Mediterranean fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 83: 1949-1953.

SPSS. 1993. SPSS for Windows release 6.0. SPSS, Inc. Chicago, IL.